PROSPECTING FUNGI FOR METHANE BIOFILTRATION REVEALS HIGH-EFFICIENCY CAPTURE BY DRIED MYCELIA (NECROMASS)

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DEDICATION

I dedicate this thesis to my wife Jillian Reilly for her love, encouragement and help. I also want to dedicate this work to my family in Malaysia for their love and support over the years.

Abstract

Fungi can improve biofiltration of hydrophobic pollutants, not via oxidation (as for bacteria/archaea) but instead by improving capture, a common rate-limiting step in bioreactors. Here, we prospected 11 candidate fungi alongside native biofilm preparations and relevant controls (e.g. activated carbon) for their efficacy capturing methane, a common hydrophobic pollutant that can be 'scrubbed' using gas-phase biofilters. Using a batch incubation system modified for various substrates, we found that *Ganoderma sessile*, a species in the *Ganoderma lucidum sensu lato* complex, performed best in single-strain trials, removing 79% of the amount of methane removed by activated carbon (61% of total injected). Building on this, we tested other *Ganoderma* species (*G. applanatum*, *G. meredithae*) and found comparable efficacies. The advantages of *Ganoderma* isolates, however, were lost relative to *Pleurotus* species and native colonizers when pre-colonized wood substrates were deployed in the field, irrespective of where they were deployed. This likely relates to a stress-tolerant (S-selected) rather than competitive (C-selected) life history strategy, where *Ganoderma* species are outcompeted in less stressful environments. Given this, we tested an alternative way to use *Ganoderma* for filtration – in dried form. Using protocols for culinary and biomaterial applications, we re-tested several fungi, including *Lentinula edodes* 'shiitake.' In these trials, we found surprisingly high efficacy (84%) relative to activated carbon, with *Ganoderma* mycelia again the top performer. These results suggest that *Ganoderma* species, fungi with long histories in cultivation, medicine, and bio-materials might best be utilized for biofiltration in dried form, a presentation likely more effective in field conditions and also potentially more amenable for biofiltration indoors.

TABLE OF CONTENTS

List of Tables v
List of Figures vi
1. Literature Review 1
    1.1 Logic of My Biofilter Focus 1
    1.2 Biofilters in Applications 2
    1.3 Biofilter Successes and Limitations 3
    1.4 Microbial Basis for Biofilter Performance 4
    1.5 A Case for Fungi in Gas-phase Biofilters 5
    1.6 The Frame for My Overarching Hypotheses 6
    1.7 My Research Objectives 6
2. Prospecting Fungi for Methane Biofiltration Reveals High-Efficiency Capture by Dried Mycelia 7
    2.1 Introduction 7
    2.2 Materials & Materials 9
        2.2.1 Vial System Set Up 9
        2.2.2 Methane Capture Analysis – Gas Chromatography 9
        2.2.3 Fungal Isolate Surveys 10
        2.2.4 Assessing Performance in Competitive Field Conditions 11
        2.2.5 Assessing Performance of Dried Fungal Materials 13
        2.2.6 Statistical Analyses 13
    2.3 Results 14
        2.3.1 Methane Capture by Fungi in Pure Culture 14
        2.3.2 Field Trials: Performance in the Face of Competitors 17
        2.3.3 Methane Capture by Dried Fungal Material 17
3. Discussion 20
4. Reference list 23

LIST OF TABLES

Table 1 Survey of fungal isolates for methane (CH_4) capture efficiency. Means and standard deviations of methane capture by non-inoculated aspen blocks, activated carbon, 6 white rot fungal strains and 2 brown rot (n=70, per treatment) 16

Table 2 Difference in methane (CH_4) capture among fungi in the genus *Ganoderma*. Means and standard deviations of CH_4 capture by surface covered with wax (to assess agar absorption), sterile semi-solid agar, and agar colonized by a mycelial mass of *Ganoderma* spp. (n=8, per treatment) 17

LIST OF FIGURES

Figure 1.A Sealed serum vial (10mL) as batch biofilter 11

Figure 1.B Assembly of fungal inoculated wood (solid medium) for gas capture 11

Figure 1.C Assembly of fungal inoculated agar (semi-solid medium) for gas capture 11

Figure 1.**D** Methane (1000ppm or 1000 mg/L) was injected into the vial using gas tight syringe 11

Figure 2.A Graph shows methane capture of field trial samples buried in soil at the atrium 19

Figure 2.B Graph shows methane capture of field trial samples buried in soil at the fungal garden..... 19

Figure 3 Mean capture efficiency (± standard deviation) of dried fungal mycelium compared with sand and activated carbon (positive control, 98% capture) 20

1. LITERATURE REVIEW

1.1 Logic of my Biofilter Focus

Biofiltration is a pollution control technique using bioreactors that contain living material to capture and degrade pollutants (Cooper & Alley, 2011). In the biofilter, naturally-occurring bacteria and fungi are immobilized on filter media to consume polluting compounds in wastewater and gas-phase effluent (Nicolai & Janni, 2001). Municipal, industrial and agricultural wastewater that contains soluble pollutants can be treated by biofilters designed as slurry bioreactors. (Lewandowski & Beyenal, 2003). Typically, biological treatment processes of the pollutants occur in the activated sludge of the slurry bioreactor after solids in wastewater are separated by physical straining using membrane processes. (Lewandowski & Beyenal, 2003, Bouwer & Crowe, 1988). In the activated sludge, microorganisms are inoculated with the suspension to form flocculation that effectively degrades the polluting compounds (Ofiteru et al., 2010).

In addition to aqueous phase pollutants, biofilters are also widely used to remove hazardous air pollutants, odorous gases and volatile organic compounds from gas-phase effluent (Akdeniz, Janni & Salnikov, 2011; Daugulis and Boudreau, 2003). In gas-phase biofilters, contaminated air is forced into the system under pressure and passed through wetted filter bed or circulating liquid that contains the microbial biomass (Cooper & Alley, 2011; Nicolai & Janni, 2001; Devinny, Deshusses & Webster, 1999). Essentially, the pollutants are captured by the biofilms in which the microbial biomass grows following their absorption rates before mass transferred into microbial cells for biodegradation (Devinny, Deshusses & Webster, 1999). Current designs for gas-phase biofilters mainly target pollutants with high solubility and low molecular weight emitted from industrial and agricultural settings, including alcohols, aldehydes, H_2S, and NH_3. The success rate of biofilters in treating contaminated air corresponds with the polluting compounds that follow increasing Henry's coefficient as they more easily dissolve into the liquid films in the biofilters. However, not all gaseous pollutants are highly soluble and many have issues being degraded in conventional biofiltration systems (Cooper & Alley, 2011; Devinny & Ramesh, 2005). Gaseous pollutants such as hydrophobic volatile organic compounds (VOCs), aromatic and aliphatic hydrocarbons remain key issues for biofilters due to their low solubility in the liquid phase and limited mass transfer into the microbial biofilms formed over the filter media. Hence, the removal efficiency of the biofilter is hindered by the capture ability

rather than the oxidation rate of the microorganisms (Limbri et al., 2013; Stresse et al., 2005).

This rate limitation in capture rather than oxidation has not been intuitive to microbiologists, who have devoted far more research attention to the oxidation of these pollutants. That oxidation step is the 'sink' but the knowledge gap lies 'upstream' in the capture. Therefore, my thesis aimed to test whether biofilters can improve the performance of biofilters by increasing capture efficiency of hydrophobic VOCs, especially CH_4, by assessing the organisms best known to improve capture in these systems - fungi.

1.2 Biofilters in Application

Since the early 1980s, biofilters have emerged as some of the most widely used bioremediation techniques because of their efficiency, low-cost and versatility (Devinny & Ramesh, 2005). Biofilters are a pollution control technology to treat wastewater and gas phase effluent using bioreactors that contain microorganisms (Cooper & Alley, 2011). The chief principle of the biofilter is to deploy microorganisms that are immobilized on filter media to consume liquid or gas-phase polluting compounds as carbon and energy sources. By doing so, polluting compounds are converted into innocuous end products before releasing into the environment (Cooper & Alley, 2011; Nicolai & Janni, 2001).

In wastewater treatment, biofilters have long been utilized in treatment plants to recycle water that can be used for various purposes (Rocher et al., 2012). Aqueous-phase biofilters designed as slurry bioreactors with activated sludge proved effective in removing excessive soluble compounds such as phosphorus, nitrate, heavy metals, and fossil fuel byproducts from agricultural, industrial and municipal wastewater (Chaudhary et al., 2003). The basic mechanisms of the aqueous phase biofiltration system are to first remove solids using membranes or granular materials as filters and then to biologically treat the wastewater in the activated sludge (El Hannadeh et al., 2017; Chaudhary et al., 2003; Lewandowski & Beyenal, 2003). The activated sludge in the system contains a flocculation of microbial biomass formed by the microorganisms suspended in the solution, providing effective surface area and sufficient biomass to consume and degrade the polluting compounds in the wastewater (Fleming & Wingender, 2003). In addition, biochar and granular activated carbon are commonly added to the sludge as filter media and as solid surfaces in which the microorganisms can attach and develop their biofilms (Lebrero et al., 2011; Chaudhary et al.,

2003). These can improve contaminant removal, but they are also expensive, particularly in the case of activated carbon at the loadings typical at an industrial treatment scale.

In addition to aqueous-phase systems, gas-phase biofilters have increasingly been applied by industries to control and improve air quality. The main targets for gas-phase biofilters consist of odorous gases, volatile organic compounds (VOCs), and hazardous air pollutants (HAPs) (Deshusses & Johnson, 2000). In the gas-phase biofilter, the contaminated airstream is forced into the system and passed through wetted filter media or circulating liquid that contains a healthy population of microorganisms. As air flows through the system packed with wetted filter media along with biomass of the microorganisms, the soluble polluted gaseous compounds and oxygen in the airstream are transferred on the wetted media into the immobilized biofilms, subsequently, degraded by the microorganisms, metabolically (Cooper & Alley, 2011). Generally, the microorganisms grow and attach on filter media such as soil, peat, compost, and wood chips (Janni et al., 2009; Nicolai & Janni, 2001). There are three common types of biofiltration for gas-phase effluent treatment: biofilter, biotrickling filter, and bioscrubber. The differences between these systems are based on how the microorganisms are inoculated and liquid phase that carries the nutrients and soluble pollutants through the system (Devinny, Deshusses & Webster, 1999). Biofilters, once developed, contain a relatively fixed biomass of microorganisms on the filter media with a fixed semi-aqueous phase, whereas biotrickling filters require recirculating liquid phase to provide nutrients and supplements to the fixed microorganisms growing on the filter media (Spennati et al., 2017). Lastly, bioscrubber is a type of biofiltration system that suspends microorganisms in flowing nutrients and media to treat an inflow polluted airstream (Bravo et al., 2017; Liu et al., 2016).

1.3 Biofilter Successes and Limitations

Other researchers have shown that gas-phase biofilters are most efficient when treating highly soluble organic compounds with low molecular weight and simple chemical structure, such as alcohols, aldehydes, and ketones as well as agricultural waste gases like H_2S, and NH_3 (Devinny & Ramesh, 2005). Typically, the liquid phase in the biofilter helps gaseous pollutants from the airstream be absorbed into the wetted filter media, where the microbial biomass is attached. This mechanism is driven by the pollutant solubility and Henry's law constant (Yaws & Yang, 1992). Meanwhile, the mass transfer rate of the gaseous pollutants into

microbial cells depends on the physical parameters of the system, such as surface area of the biofilms, turbulence and aeration in the bioreactor as well as concentration of the polluting compounds (Lewandowski & Beyenal, 2003; Zhu et al., 2001; De Beer et al., 1996).

While biofilters are a success story in some cases, using biofilters to treat hydrophobic VOCs such as benzene, toluene, ethylene, xylene, hexane, and styrene has not been as consistent as treating soluble compounds (Zehraoui et al., 2014). Hydrophobic compounds including aromatic and aliphatic hydrocarbons tend to be nonpolar and do not dissolve well in water phase (Cheng et al., 2016). Although the development and formation of microbial biomass, extracellular matrix, and byproducts in the liquid phase of biofilter bed can improve the solubility of hydrophobic VOCs (Yeo et al., 2015), the limited mass transfer of hydrophobic VOCs from the airstream into microbial biofilms remains an important challenge in biofilters targeting hydrophobic contaminants, a common goal in these systems (Lebrero et al., 2014; Singh 2006; Delhoménie & Heitz, 2005).

1.4 Microbial Basis for Biofilter Performance

Generally, naturally-occurring bacteria and fungi are the two major microbial groups used in biofilters to facilitate the degradation of pollutants in wastewater and gas-phase effluent (Devinny & Ramesh, 2005), and there are some differences between bacterial and fungal biofilters in terms of their microbial characteristics, growth conditions, and the target pollutants to be degraded (Estrada et al., 2013). For instance, bacteria have gained much more attention for their ability to propagate in the biofilters quickly. In addition, bacterial biofilters exhibit high microbial diversity and robustness in both gas- and aqueous-phase biofiltration systems. In addition, many studies have shown that bacterial biofilters are versatile in treating a wide range of pollutants such as styrene, methane, ammonia, and many odorous gases. Recently, a few studies also indicated that hydrocarbons derived from gasoline and diesel can be successfully removed by bacteria in biofilters (Portune et al., 2015; Estrada et al., 2013).

Despite the versatility and robustness of bacterial biofilters, their performances deteriorate in the environment with low moisture environment, low pH and limited nutrient sources (Estrada et al., 2013; Ralebitso-Senior et al., 2012; Kennes & Veiga, 2004). Fungi, on the other hand, have been reported to tolerate undesirable environments with low moisture content, low pH and low nutrients availability (Vergara-

Fernandez et al., 2010). Furthermore, biofilters dominated by fungal biomass have shown to improve the biodegradation of hydrophobic VOCs in lab scale (Rene et al., 2012; Kennes & Veiga, 2004). However, the current trend in full-scale biofiltration studies mainly focuses on the impacts of the heterogenous biofilms developed by a collective microbial community in biofilters, rather than specific functionality or mechanisms provided by certain fungi or bacteria (Ralebitso-Senior et al., 2012; Strickland & Rousk, 2010). Therefore, the basis for the fungal role among other microorganisms in the biofilms active in gas-phase biofiltration are often neglected.

1.5 A Case for Fungi in Gas-phase Biofilters

Recently, biofilters dominated with fungal biomass have shown promising results in the degradation of hydrophobic VOCs (Morales et al., 2017; Revah et al., 2011). Apart from the diverse enzymatic activities and the resilience in environmentally-stressful conditions such as low moisture contents and low pH values, studies suggest that fungi can facilitate hydrophobic VOCs abatement by forming hydrophobic aerial hyphae (Vergara-Fernandez et al., 2016; Jorio et al., 2009; Baldrian, 2006). The hydrophobicity of aerial hyphae is caused by hydrophobic proteins known as hydrophobins that form on the surface of the hyphal strands to allow filamentous growth into the airstream (Smits et al., 2003; Wosten et al., 2000; Wessels, 1997). Hydrophobins are secreted by fungi and self-assemble on the interface of air-liquid to reduce surface tension on the hyphae to promote growth towards nutrients and air (Wosten et al., 2000). Many studies have suggested that such hydrophobicity and aerial hyphae might play important roles in improving the sorption of hydrophobic gases (Lebrero et al., 2014; Estevez et al., 2005). Furthermore, fungal aerial growth integrates into complex networks occupying the free aerial space and colonizing the filter media that increase effective surface area by volume of the filter media (Cheng et al., 2016; Estrada et al., 2013; Revah et al., 2011).

Despite the potential for utilizing fungi to treat hydrophobic VOCs, fungi are not often harnessed as inoculum in biofilters and often overlooked as participants, especially when treating CH_4 (Lebrero et al., 2016). According to the EPA, CH_4 is one of the most potent greenhouse gases as it has 28 times more global warming potential than CO_2 and that most CH_4 emissions are caused by human-related activities (USEPA, 2017). Therefore, biological treatment of CH_4 have become a focus to mitigate the release of such gas into the atmosphere. However, the characteristic low solubility (water solubility 2mg/L) of CH_4 has been a major

problem to the removal efficiency in biofilters (Cookney et al., 2016; Lee et al., 2015). Although studies have discovered CH_4-oxidizing archaea and bacteria (methanotrophs) because of their abilities to produce methane monooxygenases (MMOs) to metabolize CH_4, CH_4 oxidation in biofilters has been mainly affected by the capture efficiency rather than oxidation rates (Chauhan et al., 2012; Nordlund et al., 1992). The hydrophobicity of CH_4 prevents dissolution of the gas into the wetted filter media in conventional biofilters as well as limits the mass transfer rate of the gas into microbial extracellular matrix and biofilms (Peng et al., 2006, Bruusgaard et al., 2010).

1.6 The Frame for My Overarching Hypotheses

For my work, I assumed that low capture efficiency would result in limited available CH_4 in the biofilter media for methanotrophs to oxidize it (Oliver & Schilling, 2016). Research has recently verified that fungal mycelia provide surface properties that improve capture efficiency of hydrophobic VOCs, subsequently integrating the gases into microbial biofilms (Morales et al., 2017; Lebrero et al., 2014). Therefore, I hypothesized that by exploring and exploiting natural diversity among fungi in their abilities for CH_4 capture, I might better guide strain selection for use in real-world biofiltration conditions.

1.7 My Research Objectives

The research aims were to accomplish the following:

1. Develop a batch system reactor system to reliably compare gas capture capacities among test fungi.
2. Screen fungal strains using these reactors to rank fungi by their capture efficiency of hydrophobic CH_4.
3. Use these rankings in pure culture to guide a field trial to challenge top performers in mixed culture conditions.
4. Assess/compare capture performances of dried fungal materials, as an alternative ‘presentation’ of the fungi.

2. PROSPECTING FUNGI FOR METHANE BIOFILTRATION REVEALS HIGH-EFFICIENCY CAPTURE BY DRIED MYCELIA

2.1 Introduction

Concentrations of global atmospheric greenhouse gases, primarily carbon dioxide (CO_2), methane (CH_4), nitrous oxides and halogenated gases, have increased significantly as a result of industrialization (Köster et al., 2017). Among these gases, CH_4 is recognized as one of the most potent greenhouse gases due to its global warming potential (28x more than CO_2) (USEPA, 2017). Despite its relatively short atmospheric residence time (12 years), CH_4 has a stronger molar absorption coefficient for infrared radiation than CO_2 (Nelson et al., 1948). There are many human-related (anthropogenic) sources for CH_4, including enteric fermentation in livestock animals, leaks from natural gas systems, and microbial methanogenesis in landfills (NOAA, 2017). Mitigation of anthropogenic CH_4 has become a top priority (Montzka et al., 2011; Shindell et al., 2012; White House, 2014), and voluntary programs such as the Coalbed Methane Outreach Program (CMOP), Natural Gas STAR program and AgSTAR program were organized by the U.S. Environmental Protection Agency to help private sectors reduce CH_4 emissions. In cases where CH_4 is emitted at high concentrations, value can be added via combustion to generate electricity and heat (USEPA, 2014). Treating CH_4 emitted at low concentration (<5%), however, is challenging due to its incombustibility and the high costs of capture (Kim et al., 2013; Limbri et al., 2014).

Biofiltration (using biologically-active filter media) is a promising, low-cost pollution control technique for mitigation of gas emissions, including but not limited to CH_4. These gas-phase biofilters have successfully been used to co-treat odorous gas mixtures (e.g. ammonia and hydrogen sulfide; volatile organic compounds (VOCs)) generated in landfills, mining operations, and livestock housing (Devinny et al., 1999). In these settings, biofilters are well-known to capture and degrade CH_4, and CH_4-oxidizing archaea and bacteria (methanotrophs) are ubiquitous inhabitants in biofilter media (Limbri et al., 2014), producing methane monooxygenases (MMOs) as part of microbial surface communities (biofilms) (Nordlund et al., 1992). Oxidation by methanotrophs is likely the principle rate-limiting step in passive-flow and biotrickling biofilters used in landfills and coal mine ventilation air, where 80-100% reductions of CH_4 are common (Dever et al., 2007; Lebrero et al., 2014). Rates of CH_4 oxidation in these low-flow rate

biofilters can be further improved by bacterial augmentation and biostimulation with nutrient additions to promote the growth of methanotrophs (Lee et al., 2009; Nikiema et al., 2005; Menard et al., 2014).

As gas flow rates in a biofilter increase and residence times decrease, however, the limits on CH_4 oxidation will shift from oxidation rates to capture efficiency. This rate limitation by gas capture, not degradation, is a common issue in forced emission systems such as confined animal houses (Oliver and Schilling 2016), and it limits the adoption of biofilters in any system with active exhaust or recirculated air, including indoor spaces. In these higher-throuphput systems, mass transfer issues arise for CH_4 (water solubility 2 mg/L) exiting the gas phase and entering a semi-aqueous biofilm, a transition governed in part by Henry's Law and exacerbated by short (<5 sec) residence times (Peng et al., 2006; Bruusgaard et al.,2010). As a result, the adsorption rate of CH_4 by the collective microbial extracellular matrix and the absorption rate by the microbial cells decrease, hindering the performance of a gas-phase biofilter (Chen and Hoff, 2009; Melse and Van der Werf, 2005; Nikiema and Heitz, 2009). These capture issues create an issue that would likely be best addressed by focusing more broadly on biofilm communities rather than methanotrophs, alone. This has likely not be intuitive to microbiologists working in these engineered systems, and perhaps for this reason, the capture-oxidation dynamic in biofilters has rarely been exploited.

One avenue that has promised improvements in biofilter CH_4 mitigation by improving capture is inoculation with filamentous fungi. Fungi are commonly applied in bioremediation strategies targeting hydrocarbon-rich petroleum spills and various phenolic/ring compounds (Norton 2012, Brown & Peake, 2006; Vigueras et al., 2008; Taylor & Stamets, 2014), and in gas-phase biofilters, inoculating fungi has been shown to improve mitigation of hydrophobic pollutants such as benzene, toluene, and hexane (Elsgaard 2000; Vergara-Fernandez et al., 2010; Revah et al., 2011; Lebrero et al., 2012; Estrada et al., 2013; Lan Zhao et al., 2014). Fungi do not typically produce methane monooxygenase (MMO) to degrade methane, but they do improve the capture of methane. Fungi capture hydrophobic gases efficiently due, at least in part, to the growth of aerial filamentous hyphae that increase the surface area and to a class of hydrophobic proteins (hydrophobins) that cover fungal hyphae. Amphiphilic hydrophobins assemble on fungal hyphae and reduce surface tension at the aqueous-gas interface, enabling growth of aerial hyphae (Linder et al., 2005; Smits et al., 2003; Bayry et al., 2012; Wosten, 2001; Wosten et al., 1999). These hyphae provide a conduit that extends down into a collection of hyphae (mycelium) embedded within

biofilms rich in bacteria and archaea, thus improving adsorption of CH_4 and other compounds and facilitating their delivery and distribution within a microbial community. The hydrophobicity of fungal mycelia has also been shown to be higher than in spores (Chau et al. 2009) and to increase in the presence of hydrophobic gases (Vergara-Fernandez et al., 2006; Scholtmeijer et al., 2001). These two facets would likely improve biofilter performance (Nguyen et al., 2017; Liu et al. 2016), but to our knowledge, only spores in pure culture have been tested for side-by-side efficacy among fungal species (Oliver and Schilling 2016).

To address this gap, we screened mycelial CH_4 capture among fungal strains grown in various culture conditions, and then used this information to guide tests in more complex biofilm communities. We used strain performance similarly to guide tests using dried mycelia – desiccation would be a typical abiotic stress in a forced-flow biofiltration system, and drying techniques are commonly used when using fungi in biomaterial applications. We hypothesized that pure fungal mycelia would improve, relative to spores, the capture of CH_4 at low concentrations <5%, and that this capture efficacy would vary among strains. We also hypothesized that performance in pure culture would not reflect performance in the field or after desiccation.

2.2 Materials & Methods

2.2.1 Vial system set up

To assess methane capture in living biomass of test fungi, relative to other various substrates, as well as assessing capture in dried fungal material, we constructed a small-scale batch biofilter system (**Figure 1A**). Serum vials (10mL) sealed with two-leg lyophilization vial stoppers and perforated aluminum capsules (Wheaton, NJ) were used as batch vessels to contain semi-solid media overgrown with fungal hyphae (**Figure 1B**), wood blocks colonized and overgrown with fungal hyphae (**Figure 1C**), and dried fungal mycelia.

2.2.2 Methane capture analysis - Gas chromatography

Methane capture in the batch vessels was measured after various residence times as the difference between inlet and outlet concentrations (as % removal). To determine methane concentration, gas samples were extracted via syringe and measured using capillary gas chromatography (GC) with a 0.55mm x 30m column (Agilent Technologies, Santa Clara, CA, USA) and using flame ionization detection (FID), HP

5890 Series II (Hewlett Packard, Saint Paul, MN, USA). The injection temperature and the oven temperature were maintained at 150ºC and 50ºC, respectively. Head pressure was maintained at 20 psi, and flow rate was 20 scmm (standard cubic meter per minute). The retention time for methane in this configuration was 1.4 minutes.

In this set-up, we measured methane capture by non-inoculated substrates and fungal-inoculated substrates, an approach that allowed us to track any methane generation and/or emissions. We validated zero concentration of hydrocarbons and methane in vials by sampling 1mL headspace gas from sealed serum vials containing fungal treatment, semi-solid agar media covered with surface mycelia, field samples or dried fungal mycelia using a gas-tight syringe (Hamilton Co., Reno, NV, USA). To assess methane capture by non-inoculated (fungus-free) substrates added to the vials, 5mL methane (1000ppm or 1000mg/L) from a calibrated methane gas cylinder (CalGas, Calgary, Alberta, CA) was injected into the vial for 15 minutes. This was duplicated with samples inoculated with fungi. Headspace gas was sampled by using a gas tight syringe and measured using GC-FID (**Figure 1D**). Pure sea sand (20 mesh) and activated carbon were used as inert and high-affinity media, respectively, to benchmark the efficiencies of our fungal treatments.

2.2.3 Fungal isolate surveys

To screen methane capture ability among wood-degrading fungal species with distinct nutritional modes, 6 white rot (lignin-degrading) fungi and 2 brown rot (more carbohydrate-selective) fungi were tested in using the batch set-up. The white rot fungi included *Pleurotus eryngii* (King oyster), *Pleurotus ostreatus* (Grey Dove™), and *Pleurotus pulmonarius* (Italian oyster) cultures that were isolated from the sawdust spawns obtained from Field & Forest Products, Inc. (Peshtigo, WI, USA), as well as *Ganoderma sessile*, a species in the *Ganoderma lucidum sensu lato* complex (UMN MN30, Forest Pathology Culture Collection, University of Minnesota), *Trametes versicolor* (MAD 677R Madison, WI, USA), and *Irpex lacteus* ATCC 11245 (American Type Culture Collection, Manassas, VA, USA). For brown rot isolates, we used *Gloeophyllum trabeum* (ATCC 11539) and *Wolfiporia cocos* (ATCC 1112). The fungi were inoculated on quaking aspen (*Populus tremuloides*) blocks (1 cm x 1.9 cm x 0.6 cm) in modified soil-block microcosms (ASTM D 1413, 2007; Song et al. 2012) and allowed to develop for 14 days at 25ºC in the dark (**Figure 1B**).

The efficacy of *Ganoderma* spp. in an initial screen prompted follow-up assessment among *Ganoderma* strains. In this experiment, we used 2% malt extract agar (w/v) (BD Difco™, Fisher Scientific, NH) as semi-solid culture media rather than solid wood blocks in order to focus dynamics on fungal hyphae, given the capture efficacy of non-inoculated wood in the first trial. First, we pipetted 5 mL of well-homogenized media into serum vials and covered with aluminum foil. Serum vials were then sterilized in an autoclave at 121ºC and 110kPa for 20 minutes. Cooled serum vials with solidified agar were inoculated with *G. sessile*, *Ganoderma applanatum* (UMN MN15) or *Ganoderma meredithae* (UMN FL50, Forest Pathology Culture Collection, University of Minnesota) and allowed to develop for 7 days at 25ºC in the dark prior to injections with methane.

2.2.4 Assessing performance in competitive field conditions

Given that fungi vary in their abilities to combat other bacteria and fungi, and that the wood-inhabiting microbes are ubiquitous in soils and mulch, we matched our experiments in the lab with an experiment in non-sterile field conditions using only the strains that were non-plant pathogenic and native to

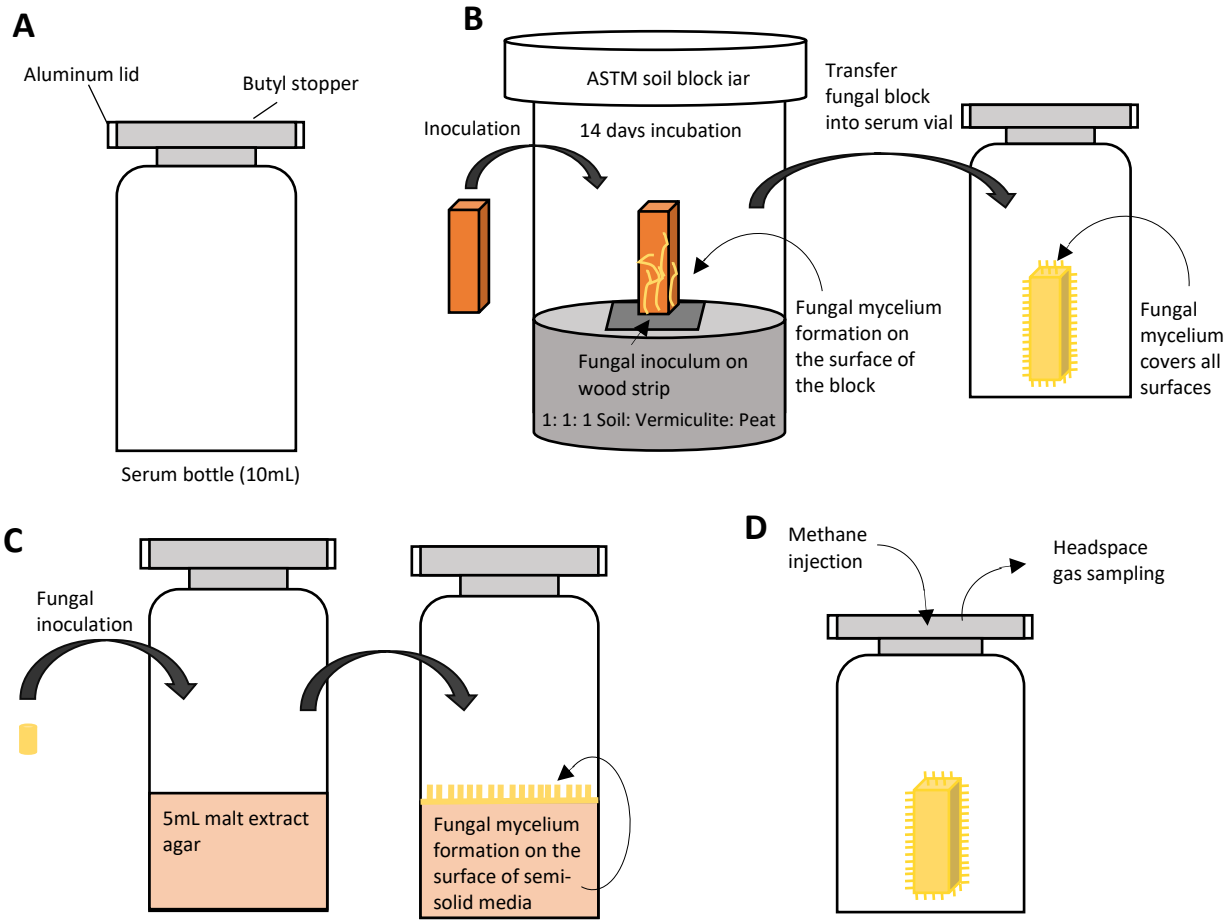


Figure 1. Components of a small-scale gas capture efficacy test. **A**. Sealed serum vial (10mL) as batch biofilter, in which efficacy of gas capture over a specific residence time in the vials was calculated as percent removed as ($\frac{C_{inlet}-C_{detected}}{C_{inlet}}$) x 100% using multiple injection (inlet) concentrations (C). **B**. Assembly of fungal inoculated wood (solid medium) for gas capture: Sterile aspen blocks were colonized in soil-block microcosms with test fungi for 14 days, harvested, placed in serum vial, and sealed with air tight crimper prior to methane injections. **C**. Assembly of fungal inoculated agar (semi-solid medium) for gas capture: Fungi were inoculated atop 5mL malt extract agar, incubated for 7 days, and injected with methane, as in wood samples. **D**. Using gas tight syringe, methane (1000ppm or 1000 mg/L) was injected into the vial to incubate for 15 minutes. 1mL headspace gas was sampled and measured by using GC-FID to assess methane concentration after fungal treatment.

Minnesota. To do this, we buried blocks pre-inoculated with select test fungi in the upper soil at two test locations, alongside non-inoculated substrates for comparison. Pre-colonized blocks were prepared by inoculating aspen blocks with *P. eryngii*, *P. ostreatus*, *P. pulmonarius*, *G. sessile* and *G. applanatum* in soil block microcosms containing well-developed fungal 'lawns' of hyphae, as in the lab trials. Non-colonized were developed similarly, but without fungal inoculum present, depending on soil-borne microbes to colonize. All wood substrates were harvested after 14 days and then added into litter bags prepared using 20 cm^2 pieces of nylon window screen cut in half and heat-sealed on the edges. The litter bags were buried 3-cm deep in the soil in field plots.

Two field plots on University of Minnesota campus were selected to vary the inoculum potential and community exposure of the pre-incubated wood blocks. One plot was located in an atrium in the Kaufert Laboratory, a site lacking connectivity with soils outside of the building and that was heavily mulched in years past, harboring Birdnest fungi in the *Nidulariaceae* family and Stinkhorn fungi in the *Phallaceae* family. Litter bags (n=8 per treatment) were buried in the shady southwest corner of the atrium to minimize desiccation. The second plot was a fungal 'garden' managed and operated by the University of Minnesota Mycology Club (MycoClub) and used to grow fungi for a farmer's market, including *Pleurotus ostreatus* (oyster) and *Lentinula edodes* (shiitake). Litter bags for each treatment (n=8 per treatment) were buried at the south end of the fungal garden, again to minimize desiccation. After 2 weeks of incubation in

either plot, blocks in the bags from both field sites were harvested. Upon harvesting, the blocks were transferred into the serum vials for gas capture testing, as before.

2.2.5 Assessing performance of dried fungal materials

Given that fungi can be grown in solid-state as bio-materials, using living-then-dried fungal hyphae, we tested gas capture efficacy of dried fungal sporophores and mycelial preparations. Fungal sporophores were produced by growing fungi on wheat straw as a substrate (solid-state culturing) in mushroom spawn bags (Field & Forest Products, Peshtigo, WI, USA). Three fungi were tested in this trial; *Lentinula edodes* (shiitake, Field & Forest Products, Peshtigo, WI, USA), *P. ostreatus* and *G. sessile*. Wheat straw was soaked for 8 hours and drained to dry. After packing wheat straw into the bags, the bags were sterilized in an autoclave at 121^{o}C and 110kPa for 20 minutes. Sterilized grow bags were cooled to room temperature before inoculating with fungi in a biosafety cabinet. The inoculated spawn bags were incubated 14 weeks at 25^{o}C in the dark. To induce fungal sporophore formation, bags were moved into a fruiting chamber maintained at 20^{o}C and 98% relative humidity for 2 weeks. After development and growth, fungal sporophores were harvested and dried at 60^{o}C in a convection oven for 24 hours. To produce fungal surface mycelia, fungi were cultivated on top of porous cellophane (Fisher Scientific, NH) placed on semi-solid malt extract agar media (recipe as before). After 10 days, surface mycelia were removed using a spatula and were dried at 60^{o}C in a convection oven for 24 hours. Dried fungal materials derived from fungal sporophores and surface mycelia were ground using Wiley mill to pass a 20 mesh (841µm). Milled and dried fungal materials were added into serum vials to investigate methane capture efficiency, as outlined above.

2.2.6 Statistical analyses

All statistical analyses were conducted with R 3.4.0 (GNU Project). One-way analysis of variance (ANOVA) was used to assess significant difference in means of methane capture efficiency between two or more fungal treatments. Tukey's HSD (honest significant difference) was used post-hoc for all tests to determine significant differences in means of methane capture. The alpha level was set to 0.05 for all analyses.

2.3 Results

2.3.1 Methane capture by fungi in pure culture

Fungal colonized wood and agar substrates, in both cases forming visible aerial hyphae that would provide surface area for interacting with the passing airstream. In the case of the wood substrates removed from cultures of fungi and exposed to methane in batch, the isolate screening trial revealed variable capacities among the fungi to capture methane. Some were below the capture efficiency of the control (26%) (**Table 1**), due either to plugging of wood pores by fungal growth or to production of methane directly by the fungi tested. Among the fungal treatments demonstrating net capture, *Ganoderma sessile* was the top performer, capturing an average of 61% of injected methane. This was statistically better than the wood-only control, and approached the performance of activated carbon that had a methane capture efficiency of 79%.

Testing the efficiency of *Ganoderma sessile* along with two other *Ganoderma* species (*G. meridethae, G. applanatum*) using semi-solid malt extract agar media (MA) indicated that *Ganoderma* species may be broadly well-suited capturing methane (**Table 2**). After 10 days of incubation, fungal mycelium had grown to cover the entire surface of the agar in plates, again with profuse aerial hyphae. *Ganoderma applanatum*, *G. sessile* and *G. meredithae* yielded similar positive results in this system, with methane capture efficiencies of 36%, 35%, and 34%, respectively. These capture efficiencies were significantly higher than those of the controls, but were lower than those in wood in the previous trial, perhaps due to the difference in the total surface area/volume ratio (wood 6.39 cm^2/mL; agar 0.9 cm^2/mL). In line with this, we observed less absorption of gas into agar than in wood, using paraffin wax at equal surface area/volume to replace an agar-only control. This wax volume captured 12% of the methane in our set-up, and the agar captured 26%. All *Ganoderma spp.* treatments exhibited higher methane capture when compared with the wax and control bottles, and we should note that these results could again be a combination of substrate and mycelial absorption/adsorption. Both experiments suggested a dynamic process within single strains of test fungi, but that some could achieve gas capture efficiencies similar to those of activated carbon.

Table 2 Survey of fungal isolates for methane (CH_4) capture efficiency. Means and standard deviations of methane capture by non-inoculated aspen blocks, activated carbon, 6 white rot fungal strains and 2 brown rot (n=70, per treatment).

					MeOH capture efficiency	
Treatment	Genus	Species	Rot type	Strain ID	Mean %	Standard Deviation
Aspen (Control)	N/A	N/A	N/A	N/A	26.40 a	15.40
Activated carbon	N/A	N/A	N/A	N/A	77.62 b	11.17
GLU	*Ganoderma*	*sessile*	White	UMN MN30	61.28 d	13.24
IRL	*Irpex*	*lacteus*	White	ATCC 11245	33.24 a	20.57
POS	*Pleurotus*	*ostreatus*	White	Grey Dove™, Field & Forest Products, Inc.	27.33 a	14.57
PER	*Pleurotus*	*eryngii*	White	King oyster, Field & Forest Products, Inc.	26.48 a	21.96
GTR	*Gloeophyllum*	*trabeum*	Brown	ATCC 11539	25.93 a	16.95
WCO	*Wolfiporia*	*cocos*	Brown	MD 104-5540	25.32 a	19.34
PPU	*Pleurotus*	*pulmonarius*	White	Italian oyster, Field & Forest Products, Inc.	8.86 c	21.48
TVE	*Trametes*	*versicolor*	White	MAD 677R	8.53 c,e	1.89

Note: Within each treatment, means with different letters differ significantly ($p<0.05$).

Table 2 Difference in methane (CH_4) capture among fungi in the genus *Ganoderma*. Means and standard deviations of CH_4 capture by surface covered with wax (to assess agar absorption), sterile semi-solid agar, and agar colonized by a mycelial mass of *Ganoderma* spp. (n=8, per treatment).

				CH_4 capture efficiency	
Treatment	Genus	Species	Strain ID	Mean (%)	Standard deviation
Wax	N/A	N/A	N/A	12.18 a	12.86
Control	N/A	N/A	N/A	26.36 a,b	3.84
GAP	*Ganoderma*	*applanatum*	UMNMN15	35.75 b	5.22
GLU	*Ganoderma*	*sesille*	UMNMN30	35.50 b	9.12
GME	*Ganoderma*	*meredithae*	UMNFL50	33.79 b	5.33

Note: Within each treatment, means with different letters differ significantly ($p<0.05$).

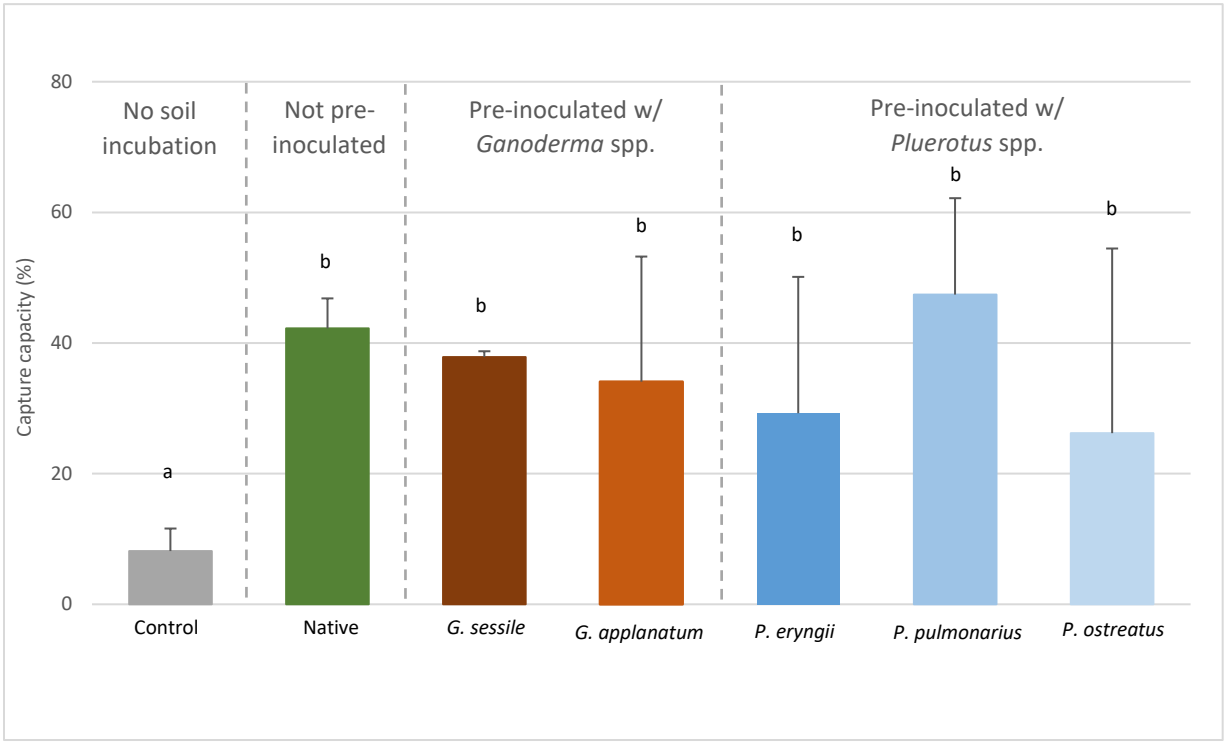
2.3.2 Field trials: Performance in the face of competitors

Knowing that efficacy varied depending on substrate variables and growth conditions, we further challenged select strains in a real-world, competitive trial by placing pre-inoculated blocks in contact with soil at two field sites. We also included a treatment with no pre-inoculation/colonization in order to assess gas capture by native strains colonizing the media, a dynamic typical in an engineered bioreactor or biofilter system as well as the dynamic relevant to any natural microbial community in soil that might capture and mitigate methane emissions. Capture efficiencies by the native biofilms established on the non-fungal treated blocks (Native) were similar in most cases with the blocks treated with one of two *Ganoderma spp*. or one of three *Pleurotus* spp. (**Figure 2 A** & **B**). There was no significant difference between treatments, but all of the wood substrates colonized by soil and/or inoculated microbial organisms had significantly greater capture efficiencies than non-colonized controls. This demonstrates the importance of a developed biofilm in capturing methane in biofilters, but it suggests that the efficacies observed in the lab may not translate in the field, where combative colonizers may overtake, dominate, and drive observed performance dynamics.

2.3.3 Methane capture by dried fungal material

In our final trials, we used dried, dead fungal biomass (necromass) rather than living mycelium, and this use of necromass resulted in the highest capture efficiencies that we observed in any of our 'living biomass' trials. Again, *Ganoderma sessile* outperformed the other strains tested, and although activated carbon remained superior (98% capture efficiency), dried *G. sessile* mycelial mass achieved 83% removal efficiency. Results are shown for both fungal surface mycelium and fungal fruiting bodies (**Figure 3**). Generally, greater capture efficiency was observed for fungal surface mycelia rather than fruiting body material, although both performed better than sand-only controls.

A


No soil incubation
Not pre-inoculated
Pre-inoculated w/ *Ganoderma* spp.
Pre-inoculated w/ *Pluerotus* spp.
Capture capacity (%)
80
60
40
20
0
a
b
b
b
b
b
b
Control
Native
G. sessile
G. applanatum
P. eryngii
P. pulmonarius
P. ostreatus

B

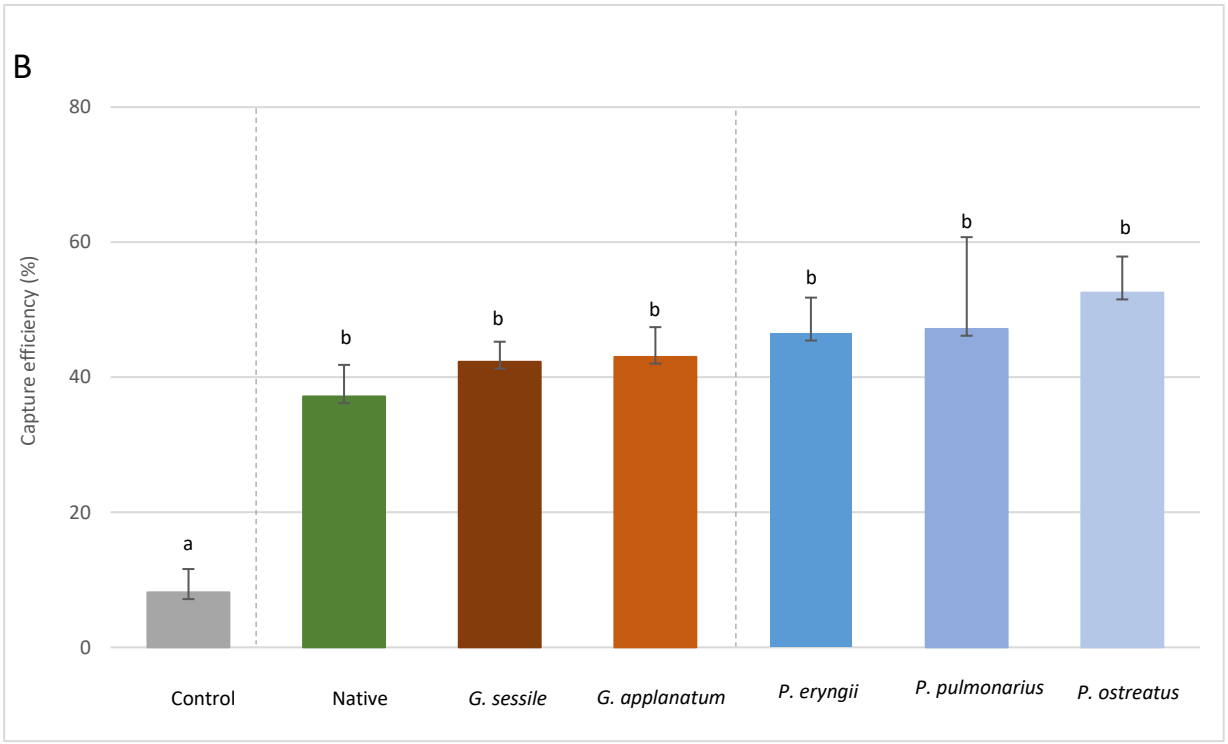

Capture efficiency (%)
80
60
40
20
0
a
b
b
b
b
b
b
Control
Native
G. sessile
G. applanatum
P. eryngii
P. pulmonarius
P. ostreatus

Figure 2. Methane capture of field trial samples buried in soil at site #1 (atrium) (A) or at site #2 (fungal garden) (B). The capture efficiencies of methane were measured in control aspen blocks (not colonized), microbial biofilms established naturally on aspen blocks when buried (Native) and biofilms established naturally on blocks pre-inoculated in the lab with fungi from *Ganoderma* and *Pleurotus* genera, then buried. Different letters note statistically different means (α=0.05).

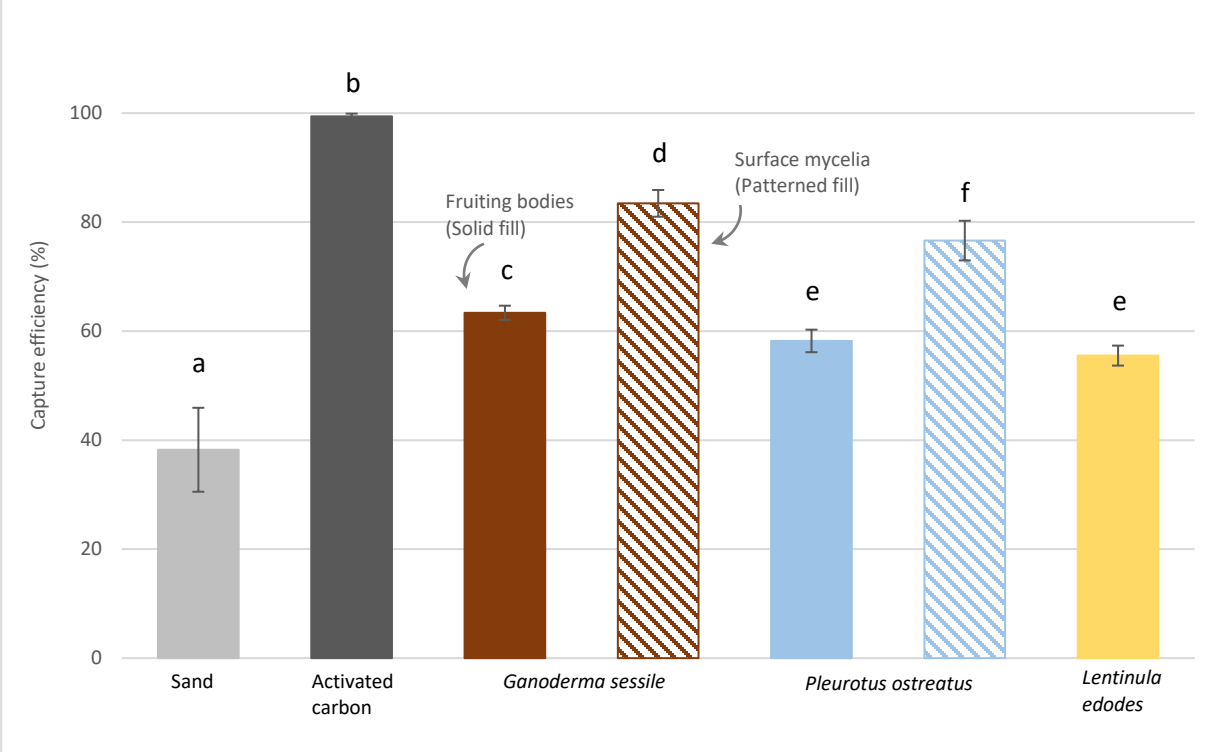


Figure 3. Mean capture efficiency (± standard deviation) of dried fungal mycelium for *Ganoderma sessile*, *Pleurotus ostreatus* and *Lentinula edodes*, compared with sand and activated carbon (positive control, 98% capture). Dried fungal mycelia were derived from fungal sporophores (fruiting bodies; mushrooms or conks) or surface mycelia grown on solid media. Different letters note statistically different means (α=0.05).

3 Discussion

The test system in this experiment, using sealed glass serum vials for batch incubations, proved useful in screening different fungi and substrates, consistently. In addition, the materials required for the vial set-up were inexpensive and there was minimal space required to operate the experiment. Fungal hyphae that formed mycelia on substrate surfaces in many cases offered an effective surface area for CH_4 adsorption and capture potential, as has been shown previously (Oliver & Schilling, 2016; Vergara-Fernandez et al., 2006; Scholtmeijer et al., 2001). Designs using semi-solid substrate such as malt extract agar media were less effective than wood substrates, but there was also lower porosity for adsorption of gas molecules in the non-colonized agar, an observation in line with previous work (Barrett-Lennard & Dracup, 1988). Comparatively, wood blocks with or without fungal treatment exhibited higher CH_4 capture ability, perhaps in part due to surface area and porosity of the blocks (Donaldson et al., 2015; Akedeniz et al., 2011). Gas capture efficacy was higher in non-treated wood blocks than in fungal treatments in some cases, perhaps because of the higher ratio of available pores for gas molecules to be adsorbed. In fungal treated wood blocks, fungal hyphae likely infiltrated wood cells via cell lumens to initiate decay (Blanchette et al., 1997), decreasing available pore space for the adsorption of gas molecules. In addition to this 'clogging' potential, saprotrophic fungi have also been implicated in producing methane as they degrade dead woody biomass (Lenhart et al., 2012; Liu et al., 2017). These factors may have combined to yield capture values for fungi that were less than those in the non-colonized controls.

Although some fungi failed in the microcosm vials, several fungi demonstrated significant capture potential in this set-up, most notably *Ganoderma* species. *Ganoderma sessile* outperformed other fungi in the initial screen, and in the follow-up trial, the mycelia of 3 *Ganoderma* species (*G. sessile*, *G. applanatum* and *G. meredithae*) inoculated on semi-solid culture media similarly had high capture efficacies. The results suggest generally that fungal mycelia can be effective in capturing hydrophobic gas such as CH_4 in the atmosphere, perhaps related to aerial hyphae development and to hydrophobins that allow fungal hyphae to extend into the airstream and cause fungal biomass to be hydrophobic (Chau et al., 2009; Smits et al., 2003; Nguyen et al., 2017; Liu et al. 2016). *Ganoderma*, specifically, is a notable 'front-runner' for use in biofiltration of gases for several reasons. 1) *Ganoderma* spp. are widespread basidiomycetes, well-known in tropical forests (Hong & Jung, 2004; Moncalvo et al., 1995; Adaskaveg & Gilbertson, 1986), but

also from temperate systems where they are common tree pathogens (e.g., Skaria et al., 1990; Knorr, 1973) and white rot type (lignin-degrading) saprotrophs of deadwood (Adaskaveg & Gilbertson, 1986). 2) The lignolytic capacity of *Ganoderma* has spawned a great deal of research focused on biotechnological utilization (Torress-Farrada et al., 2017; Cilerdzic et al., 2016), yielding an extensive literature base. 2) *Ganoderma* spp. are commonly used to manufacture solid biomaterials such as panels and packaging molds. The dried mycelia have desirable qualities such as low wettability, excellent strength, fire resistance, insulation and moldability (Bayer et al., 2009; Holt et al., 2012). 4) In eastern Asia, *G. lucidum* mushrooms (known as Lingzhi or Reishi) have long been cultured commercially in solid state for medicinal and nutritive purposes (Wu et al., 2012; Bishop et al., 2015; Gill et al., 2017; Sanodiya et al., 2009). These attributes make *Ganoderma* spp. attractive in application, perhaps using dried *Ganoderma* mycelial material for outdoor and indoor biofiltration designs.

Field trials revealed that native microbes among multiple locations could form biofilms that were at least as effective as those using targeted pre-inoculations. Selected *Ganoderma* spp. and *Pleurotus* spp. were able to develop fungal mycelia on the wood blocks prior to field trials, but this 'priority' colonization may not have been enough to establish dominance over the incubation times in the field. The failure of fungal pre-inoculation priority effects, known to steer wood-decay community development (Fukami et al. 2010), might be related to the inoculum potential in the soil (Song et al., 2015), as well as the stress-tolerant (S-selected) rather than competitive (C-selected) life-history strategies of *Ganoderma* and *Pleurotus* spp. (Boddy and Heilmann-Clausen 2008). It is likely that the successful colonizers in our field trial had similar function (functional redundancy) and similar efficacy in gas capture (Walker 1992). Typically, efficient gas-liquid mass transfer of hydrophobic CH_4 is limited in the biofilter because the gas moves from the aqueous phase into the gas phase as a function of Henry's law. However, microbial biofilms, especially fungal hyphae and mycelia from native colonizers, can play the same role as *Ganoderma* spp. on the wood blocks by increasing the contact of CH_4 and capturing the gas before transferring it into the living biofilms (Kroukamp & Wolfaardt, 2009; Holden et al., 1997).

These collective results, one showing the potential of *Ganoderma* spp. but another showing its limitations in complex microbial communities, suggests that our most important results may be with the exceptional gas capture efficiencies of dried fungal biomass. To our knowledge, this is the first study to test

dried fungal materials (fungal sporophores and surface mycelia) in gas capture trials, and we discovered an impressive capacity of dried *Ganoderma* mycelia to 'scrub' methane. When comparing capture potentials between dried fungal mycelium and laboratory grade sand, dried mycelia demonstrated higher capture efficacy perhaps due to an abundance of hydrophobic proteins (hydrophobins) (Yu et al., 2008; Huang et al., 2013). Under typical circumstances, hydrophobins remain relatively stable at temperatures below 70 ºC and can improve adsorption and diffusion rates of hydrophobic gases such as benzene, hexane and styrene into the interfacial biofilms (Liu et al., 2016; Vergara-Fernandez et al., 2006; Kennes & Veiga, 2004).

Drying fungi is an ancient practice related to food storage that has been adopted and harnessed more recently for making solid bio-based materials. The process for producing *Ganoderma* 'bricks' starts with growing the fungus on substrates such as organic and agricultural byproducts. After molding and drying the fungal mycelium covered materials, the fungal materials are applied as containers or packaging materials aiming to reduce the use of fossil fuel products such as Styrofoam (Bayer et al., 2009). Similarly to food storage, a hot-air drying process is most commonly used to insure longevity of the solid materials. For centuries, a hot-air drying process has been used for mushrooms such as *Lentinus edodes* (shiitake) and *G. sessile*, a process that improves shelf life as well as altering the aroma and flavor profiles of the mushrooms for use in cooking (Yang et al., 2017; Hayati et al., 2016; Hiraide, 2006). These same techniques to grow and dry *G. sessile* materials may alter other chemical attributes in biotechnologically-desirable ways and may be useful for developing non-living 'bio'filters for mitigation of a variety of hydrophobic volatile organic compounds (VOCs) indoors (Vergara-Fernandez et al., 2008; Rene et al., 2012).

Reference list

Akdeniz, N., Janni, K.A. and Salnikov, I.A. 2011. Biofilter performance of pine nuggets and lava rock as media. Bioresource Technology. 102(8):4974-80.

Baldrian, P. 2006. Fungal laccases - occurrence and properties. FEMS Microbiology Review. 2006 30(2):215-42.

Bouwer, E.J. and Crowe, P.B. 1988. Biological processes in drinking water treatment. Journal of American Water Works Association. 80(9): 82-93.

Bravo, D., Ferrero, P., Penya-Roja, J.M., Alvarez-Hornos, F.J., and Gabaldon, C. 2017. Control of VOCs from printing press air emissions by anaerobic bioscrubber: Performance microbial community of an on-site pilot unit. Journal of Environmental Management. 197: 287-295.

Bruusgaard, H., Beltran, J.G., and Servio, P. 2010. Solubility measurements for the $CH_4 + CO_2 + H_2O$ system under hydrate–liquid–vapor equilibrium. Fluid Phase Equilibria. 296(2): 106-109.

Chaudhary, D.S., Vigneswaran, S., Ngo, H-H., Wang, G.S., Hee, M. 2003. Biofilter in water and wastewater treatment. Korean Journal of Chemical Engineering. 20(6): 1054-1065.

Chauhan, A., Pathak, A., and Ogram, A. 2012. Composition of methane oxidizing bacterial communities as a function of nutrient loading in Florida Everglades. Soil Microbiology. 64(3): 750-759.

Cheng, Y., He, H., Yang, C., Zeng, G., Li, X., Chen, H., and Yu, G. 2016. Challenges and solutions for biofiltration of hydrophobic volatile organic compounds. Biotechnology Advances 34 (6): 1091-1102.

Cookney, J., Mcleod, A., Mathioudakis, V., Ncube, P., Soares, A., Jefferson, B., and McAdam, E.J. 2016. Dissolved methane recovery from anaerobic effluents using hollow fibre membrane contactors. Journal of Membrane Science. 502: 141-150.

Cooper, D.C. and Alley, F.C. 2011. Air pollution control: A design approach 4th edition.

De Beer, D., Stoodley, P., and Lewandowski, Z. 1996. Lidquid flow and mass transport in heterogeneous biofilms. Water Resources 30(11): 2761-2765.

Delhoménie, M.C. and Heitz, M. 2005. Biofiltration of air: A review. Critical Reviews in Biotechnology. 25(1-2): 53-72.

Deshusses, M.A. and Johnson, C.T. 2000. Development and validation of a simple protocol to rapidly determine the performance of biofilters for VOC treatment. Environmental Science and Technology. 34(3): 461-467.

Devinny, J.S. and Ramesh, J. 2005. A phenomenological review of biofilter models. Chemical Engineering Journal. 113(2-3): 187-796.

Devinny, J.S., Deshusses, M.A., and Webster, T.S. 1999. Biofiltration for air pollution control. CRC Press 1998.

Estevez, E., Veiga, M.C., and Kennes, C. 2005. Biofiltration of waste gases with the fungi *Exophiala oligosperma* and *Paecilomyces variotii*. Applied Microbiology and Biotechnology. 67(4): 563-568.

Estrada, J.M., Hernandez, S., Munoz, R., and Revah, S. 2013. A comparative study of fungal and bacterial biofiltration treating a VOC mixture. Journal of Hazardous Materials. 250-251(2013): 190-197.

Fleming, H.C., Wingender, J. 2003. The crucial role of extracellular polymeric substances in biofilms. *In* Biofilms in Wastewater Treatment: An Interdisciplinary Approach. Wuertz, S., Bishop, P., Wilderer, P. IWA Publishing, London.

El Hannadeh, A., Albalasmeh, A.A., and Gharaibeh, M. 2017. Phosphorus removal from wastewater in biofilters with biochar augmented geomedium: Effect of biochar particle size. Clean – Soil, Air, Water 45(7).

Janni, K.A., Schmidt, D.R., Goldman, A., Schaar, T. 2009. Alternative gas phase biofilter media characteristics and performance. ASABE Annual International Meeting, St Joseph, MI paper no. 097037.

Jorio, H., Jina, Y., Elmrini, H., Nikiemaa, J., Brzezinski, R., and Heitz, M. 2009. Treatment of VOCs in biofilters inoculated with fungi and microbial consortium. Environmental Technology. 5(2009): 477-485.

Kennes, C. and Veiga, M.C. 2004. Fungal biocatalysts in the biofiltration of VOC-polluted air. Journal of Biotechnology. 113(1-3): 305-319.

Lebrero, R., Rodriguez, E., Estrada, J.M, Garcia-Encina, P.A., and Munoz, R. 2011. Odor abatement in biotrickling filters: Effect of the EBRT on methyl mercaptan and hydrophobic VOCs removal. Bioresource Technology. 109(2012): 38-45.

Lebrero, R., Hernandez, L., Perez, R., Estrada, J.M., and Munoz, R. 2015. Two-liquid phase partitioning biotrickling filters for methane abatement: Exploring the potential of hydrophobic methanotrophs. Journal of Environmental Management. 151(2015): 124-134.

Lebrero, R., Lopez, J.C., Lehtinen, I., Perez, R., Quijano, G., and Munoz, R. 2016. Exploring the potential of fungi for methane abatement: Performance evaluation of a fungal-bacterial biofilter. Chemosphere. 144(2016): 97-106.

Lee, J., Yasin, M., Park, S., Chang, I.S., Ha, K-S., Lee, E.Y., and Lee, J. 2015. Gas-liquid mass transfer coefficient of methane in bubble column reactor. Korean Journal of Chemical Engineering. 32(6): 1060-1063.

Lee, S.J., McCormick, M.S., Lippard, S.J., and Cho, U-S. 2013. Control of substrate access to the active site in methane monooxygenase. Nature. 494:380-384.

Lewandowski, Z., Beyenal, H. 2003. Mass transport in heterogeneous biofilms. *In* Biofilms in Wastewater Treatment: An Interdisciplinary Approach. Wuertz, S., Bishop, P., Wilderer, P. IWA Publishing, London.

Limbri, H., Gunawan, C., Rosche, B., and Scott, J. 2013. Challenges to developing methane biofiltration for coal mine ventilation air: A review. Water, Air and Soil Pollution. 224(2013):1566.

Limbri, H., Gunawan, C., Thomas, T., Smith, A., Scott, J., and Rosche, B. 2014. Coal packed methane biofilter to mitigate for greenhouse gas emission from coal mine ventilation air. PLOS One. 9(4): e94541.

Liu, H.B., Yang, B., and Xue, N.D. 2016. Enhanced adsorption of benzene vapor on granular activated carbon under humid conditions due to shifts in hydrophobicity and total micropore volume. Journal of Hazardous Materials. 318(2016): 425-432.

Nicolai, R.E. and Janni, K.A. 2001. Biofilter media mixture ratio of wood chips and compost treating swine odors. Water Science and Technology 44, 261-267.

Nordlund, P., Dalton, H., and Eklund, H. 1992. The active site structure of methane monooxygenase is closely related to the binuclear iron center of ribonucleotide reductase. FEBS Letters. 307(3): 257-262.

Ofiteru, I.D., Lunn, M., Curtis, T.P., Wells, G.F., Criddle, C.S., Francis, C.A., and Sloan, W.T. 2010. Combined niche and neutral effects in a microbial wastewater treatment community. Proceedings of the National Academy of Sciences. 107(35): 15345-15350.

Oliver, J.P. and Schilling, J.S. 2016. Capture of methane by fungi: Evidence from laboratory-scale biofilter and chromatographic isotherm studies. Transactions of ASABE 59(6): 1791-1801.

Peng, B.Z., Chen, G.J., Luo, H., and Sun, C.Y. 2006. Solubility measurement of methane in aqueous solution of sodium dodecyl sulfate at ambient temperature and near hydrate conditions. Journal of Colloid and Interface Science. 304(2): 558-561.

Portune, K.J., Perez, M.C., Alvarez-Hornos, F.J., and Gabaldon, C. 2015. Investigating bacterial populations in styrene-degrading biofilters by 16s rDNA tag pyrosequencing. Applied Microbiology and Biotechnology. 99(1): 3-18.

Ralebitso-Senior, T.K., Senior, E., Di Felice, R., and Jarvis, K. 2012. Waste gas biofiltration: Advances and limitations of current approaches in microbiology. Environmental Science Technology. 46(16): 8542-8572.

Rene, E.R., Mohammad, B.T., Veiga, M.C., and Kennes, C. 2012. Biodegradation of BTEX in a fungal biofilter: Influence of operational parameters, effect of shock-loads and substrate stratification. Bioresource Technology. 116(2012): 204-213.

Revah, S., Vergara-Fernandez, A., and Hernandez, S. 2011. Fungal biofiltration for the elimination of gaseous pollutants from air. *In* Mycofactories. Edition 1. Bentham Science Publisher Ltd: 109-120.

Rocher, V., Paffoni, C., Goncalves, A., Guerin, S., Azimi, S., Gasperi, J., Moilleron, R., and Pauss, A. 2012. Municipal wastewater treatment by biofiltration: Comparisons of various treatment layouts. Part I. Assessment of carbon and nitrogen removal. Water Science Technology. 65(9): 1705-1712.

Singh, R.S., Agnihotri, S.S., and Upadhyay, S.N. 2006. Removal of toluene vapour using agro-waste as biofilter media. Bioresource Technology. 97(18): 2296-2301.

Smits, T.H., Wick, L.Y., Harms, H., and Keel, C. 2003. Characterization of the surface hydrophobicity of filamentous fungi. Environmental Microbiology. 5(2): 85-91.

Spennati, F., Mannicci, A., Mori, G., Giordano, C., and Munz, G. 2017. Moving bed bio trickling filters: An innovative solution for hydrogen sulphide removal from gas streams. Desalination and Water Treatment. 61(2017): 215-221.

Stresse, J. and Stegmann, R. 2005. Potentials and limitations of biofilters for methane biofiltration. Proceedings Sardinia. 3-7.

Strickland, M.S. and Rousk, J. 2010. Considering fungal: bacterial dominance in soils – Methods, controls, and ecosystems implications. Soil Biology and Biochemistry. 42(2010): 1385-1395.

USEPA, 2017. USEPA. (2015). Overview of greenhouse gases: Methane. Washington, DC: U.S. Environmental Protection Agency. Retrieved from https://www.epa.gov/ghgemissions/overviewgreenhouse-gases

Vergara-Fernandez, A., Hernandez, S., and Revah, S. 2010. Elimination of hydrophobic volatile organic compounds in fungal biofilters: Reducing start-up time using different carbon sources. Biotechnology and Bioengineering. 108(4): 758-765.

Vergara-Fernandez, A., Scott, F., Moreno-Casas, P., Diaz-Robles, L., and Munoz, R. 2016. Elucidating the key role of the fungal mycelium on the biodegradation of n-pentane as a model hydrophobic VOC. Chemosphere 157(2016): 89-96.

Morales, P., Caceres, M., Scott, F., Diaz-Robles, L., Aroca, G. and Vergara-Fernandez, A. 2017. Biodegradation of benzo[α]pyrene, toluene, and formaldehyde from the gas phase by a consortium of *Rhodococcus erythropolis* and *Fusarium solani*. Applied Microbiology and Biotechnology. Doi: 10.1007/s00253-017-8400-8.

Wessels, J.G.H. 1997. Hydrophobins: Proteins that Change the Nature of the Fungal Surface. Advances in Microbial Physiology. 38(1996): 1-45.

Wosten, H.A.B. and de Vocht, M.L. 2000. Hydrophobins, the fungal coat unraveled. Biochimica et Biophysica Acta (BBA) – Reviews on Biomembranes. 1469(2): 79-86.

Yaws, C. L. and Yang, H.-C. 1992. Henry's law constant for compound in water. In C. L. Yaws, editor, Thermodynamic and Physical Property Data, pages 181–206. Gulf Publishing Company, Houston, TX, 1992.

Daugulis, A.J. and Boudreau, N.G. 2003. Removal and destruction of high concentrations of gaseous toluene in a two-phase partitioning bioreactor by *Alcaligenes xylosoxidans*. Biotechnology Letters. 25(17): 1421-1424.

Yeo, H., An, Y., Reid, R., Rittmann, B.E., and Lee, H.S. 2015. Contribution of liquid/gas mass-transfer limitations to dissolved methane oversaturation in anaerobic treatment of dilute wastewater. Environmental Science and Technology. 49(2015): 10366-10372.

Zehraoui, A., Wendell, D., and Sorial, G.A. 2015. Treatment of hydrophobic VOCs in trickling bed air biofilter: Emphasis on long-term effect of initial alternate use of hydrophilic VOCs and microbial species evolution. Journal of the Air and Waste Management 64(10): 1102-1111.

Zhu, X., Suidan, M.T., Alonso, C., Yu, T., Kim, B.J., and Kim, B.R. 2001. Biofilm structure and mass transfer in a gas phase trickle-bed biofilter. Water Science Technology. 43(1): 285-293.